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BIOLOGICAL BULLETIN.

THE FORMATION OF THE NOTOCHORD IN THE AMPHIBIA.

HELEN DEAN KING.

A study of the mode of development of the notochord in the common toad, *Bufo lentiginosus*, and of the frog, *Rana palustris*, has brought to light certain points that have a bearing on the formation of the same structure in related groups. A vast amount of work has already been done along this line, yet a wide difference of opinion exists among embryologists regarding the origin of the notochord in the Amphibia. It is hoped that the results recorded in the present paper may help to clear up this question.

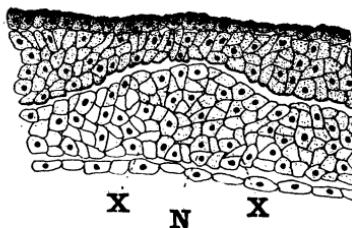
The material used was fixed in corrosive-acetic (5° glacial acetic acid), and the sections were stained on the slide with a mixture of borax-carmine and Lyon's blue as described in a previous paper (King, 11). This stain gives particularly good results when it is used on freshly preserved material, as then all of the nuclei become dark red, the ectoderm and mesoderm appear dark blue, while the yolk cells take but a pale blue tint and, therefore, are easily distinguished from the other cells. This sharp definition of the tissues was of great assistance, particularly in the study of the sections of *Bufo*. All of the drawings given in the present paper were outlined with the aid of a camera lucida.

BUFO LENTIGINOSUS.

When the circular blastopore is closing in, the mesoderm, already differentiated from the other tissues, forms a continuous sheet of small, angular, slightly pigmented cells across the dorsal wall of the archenteron. In the middle and also in the anterior part of the embryo, the mesoderm is entirely separated from the ectoderm above as well as from the endoderm beneath it. In the region just in front of the blastopore, the mesoderm is also

distinct from the ectoderm, but it is united for some distance with the cells forming the dorsal wall of the archenteron. At this stage of development there is first noticed, in the middle of the embryo, a pronounced thickening of the mid-dorsal mesoderm (Fig. 1, *N*), which extends only over a few sections at first and is continuous with the lateral mesoderm on either side. When the blastopore is nearly closed, the thickened portion of the mesoderm is cut off from the lateral mesoderm to form the notochord, the line of separation coming in at about the points marked *XX* in Fig. 1. As the embryo elongates, the forward extension of the

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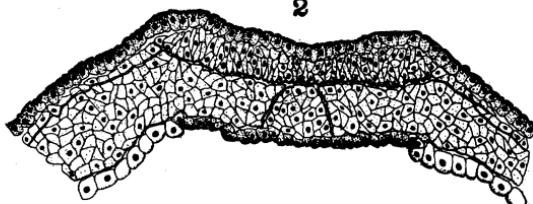
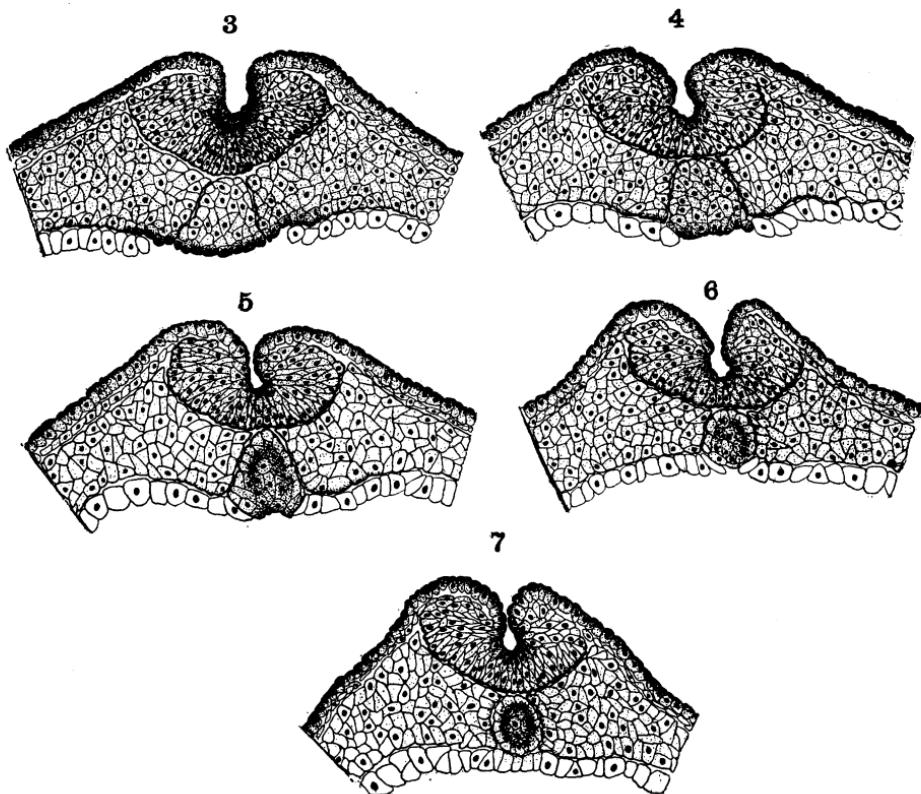


FIG. 1. Part of a medium sagittal section through an egg of *Bufo lentiginosus* in which the blastopore has begun to close. *N*, thickening of mid-dorsal mesoderm which is to be cut off at the points *XX* to form the notochord.

FIG. 2. Part of transverse section through the posterior region of an embryo in which the medullary plate has appeared.

notochord always takes place in this same way, *i. e.*, by the cutting off, laterally, of a portion of the mesodermal layer in the mid-dorsal region so that, from the beginning, the notochord is entirely separated from the ectoderm and also from the endoderm. These observations confirm the statement made in a previous paper (King, 12) that "the anterior part of the notochord is certainly mesodermal in origin."

Transverse sections through an embryo in which the medullary plate has just appeared show that, in the anterior region, the notochord is composed of a rounded mass of cells cut off entirely from the surrounding tissues, and appearing much as in Fig. 7. In the posterior region, there is, as yet, no trace of a notochord, and an unbroken layer of cells extends across the dorsal surface of the archenteron, as here the mesoderm is still



Figs. 3-7. Serial sections from the posterior to the middle region of an embryo of *Bufo lentiginosus* in which the medullary folds are closing.

united with the endoderm as in the earlier stages. In a section made a short distance behind the middle of the embryo (Fig. 2), the notochord appears as a triangular shaped chord of cells, entirely distinct from the mesodermal layer on either side, but closely connected with the cells forming the mid-dorsal wall of the archenteron. In this part of the embryo, as well as more

posteriorly, the archenteron is surrounded on its ventral, lateral and lateral-dorsal surfaces by large, rounded, faintly staining yolk cells which contain very little, if any, pigment; the mid-dorsal wall, on the contrary, is formed of a single layer of much smaller, rectangular cells which are very heavily pigmented on the side bordering the archenteron. This layer of cells, which I shall call "the dorsal plate," is broadest in the posterior part of the embryo, where, in transverse sections, it appears as a nearly straight line of cells covering about two-thirds of the mid-dorsal surface of the archenteron. More anteriorly the dorsal plate gradually becomes narrower, until it finally disappears completely in the middle of the embryo. The archenteron in front of this region is entirely surrounded by large yolk cells.

The outer cells of the dorsal plate, instead of grading into the yolk cells as one might expect, are found to be directly continuous with the lower layer of mesoderm. There is, therefore, in this region an abrupt change from the small, deeply pigmented cells of the dorsal plate to the large yolk cells which form the lateral and ventral walls of the archenteron. At no stage in the development of the embryo have I ever found any transitional stages between these two different kinds of cells. The cells of the dorsal plate resemble, in all respects, the cells forming the outer surface of the embryo, being of the same size and shape and containing about the same amount of pigment. From the results which I obtained in my study of the gastrulation of the egg of this species (King, 12), it seems highly probable that the cells composing the dorsal plate were invaginated from the surface of the egg during the formation of the blastopore, and, consequently, they have had a very different origin from the cells forming the lateral and ventral walls of the archenteron which are all derived from the yolk portion of the egg.

When the medullary folds are closing, the mesoderm in the posterior region is still connected, for a short distance, with the cells forming the dorsal wall of the archenteron, and the notochord has not yet extended into this portion of the embryo. Fig. 3 shows a portion of the section through the region where the notochord has just been cut off from the mesoderm. This section corresponds in its position in the embryo with the position

of the section of the earlier embryo shown in Fig. 2. The notochord is triangular in shape and is closely connected with the layer of cells forming the mid-dorsal wall of the archenteron. The portion of the dorsal plate directly under the notochord is cut off on either side from the rest of the layer, and to it one can, perhaps, fitly apply the term "chorda-endoderm," since it is destined to become a part of the notochord. At this stage of development, the dorsal plate is much narrower in the posterior region of the embryo than it was before the medullary folds formed (Fig. 2), and it is again found to be directly connected with the lower layer of mesoderm and not with the yolk cells forming the lateral walls of the archenteron.

In Fig. 4, a portion of a section slightly anterior to that shown in Fig. 3, the chorda-endoderm is seen to be the only portion of the dorsal plate bordering the archenteron. The other cells of the dorsal plate have united with the mesoderm, and can only be distinguished from it on account of their position and the fact that they contain somewhat more pigment. The entire dorsal wall of the archenteron, excepting the part formed by the chorda-endoderm, is here composed of large, rounded yolk cells which are evidently growing up from both sides, and thus shutting off all of the cells of the dorsal plate from bordering the archenteric cavity. More anteriorly, as shown in Fig. 5, the yolk cells of the upper wall of the archenteron are still closer together in the middle lines. In this part of the embryo the cells of the chorda-endoderm no longer form a nearly straight line at the lower edge of the notochord, but they have become an integral part of it, and most of their pigment is collected in the form of a pronounced ring around the center of the notochord.

Near the middle of the embryo (Fig. 6), the yolk cells have almost met under the notochord, which is smaller and more rounded than it is in the posterior part of the embryo. A section more anteriorly still (Fig. 7) shows that the yolk cells from the two sides of the archenteron have come together in the middle line under the notochord. As a result, the dorsal wall of the archenteron is composed entirely of a single layer of large yolk cells, and the cylindrical notochord above it is cut off entirely

from the surrounding tissues. In the head region, the relation of the tissues is practically the same as that shown in Fig. 7.

When the medullary folds have closed, there is found in the posterior region of the embryo a much narrower dorsal plate than that shown in Fig. 3, as more of the cells have been covered over by the upward growth of the yolk cells from the sides of the archenteric cavity. Anteriorly the dorsal plate grows narrower very rapidly and some distance back of the middle of the embryo the yolk cells have already come to surround the entire archenteron. By the time that the optic bulbs have formed, there is no longer any dorsal plate in the mid-dorsal wall of the archenteron and the notochord has no connection with any of the surrounding tissues.

These results show that the anterior part of the notochord in the embryo of *Bufo lentiginosus* is entirely mesodermal in origin; in the posterior part of the embryo, the greater part of the notochord is also derived from the mesoderm, but there is added to it a single layer of chorda-endoderm from the mid-dorsal wall of the archenteron. Back of the middle region of the embryo, the yolk cells grow up from the lateral walls of the archenteron and unite under the notochord, the cells of the dorsal plate thus cut off from bordering the archenteron, either unite with the notochord or are incorporated into the splanchnic mesoderm.

RANA PALUSTRIS.

In the frog, *Rana palustris*, the notochord is formed at about the same stage of development that it is in *Bufo*, namely, near the end of gastrulation when the blastopore is closing in. As in the embryo of *Bufo*, the notochord first appears in the middle region as a rounded chord of cells cut off from the mid-dorsal mesoderm, and it is separated entirely from the ectoderm and also from the endoderm beneath which forms the dorsal wall of the archenteron. At this stage in the development of the egg, the mesoderm in front of the region where the notochord has been cut off forms a solid layer of cells extending across the dorsal wall of the archenteron and entirely separated from it; the mesoderm back of the notochord also extends in an unbroken sheet across the mid-dorsal region, but in this part of the egg meso-

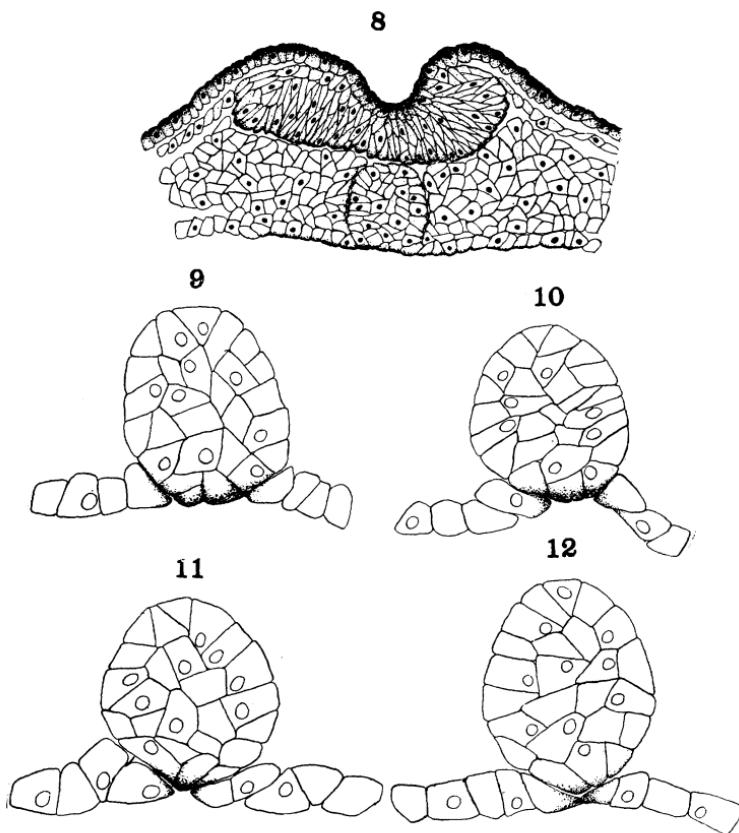
derm and endoderm are connected for a considerable distance on either side of the middle line.

In the posterior part of the embryo the cells forming the dorsal wall of the archenteron do not differ in size, shape, or in power of staining from the mesodermal cells above them, and at the sides of the archenteron they grade into the larger yolk cells forming the ventral and lateral walls. There is a comparatively narrow region in the mid-dorsal wall where the edges of the cells bordering the archenteric cavity are quite heavily pigmented; but the other cells of the dorsal wall contain about the same amount of pigment as do the mesoderm cells above them, and there is no definite dorsal plate of small, deeply pigmented, rectangular cells as in the toad embryo. I can find no evidence that any of the cells of the dorsal wall of the archenteron ever form a permanent union with the mesoderm.

When the medullary folds are beginning to form, the notochord has extended into the posterior region of the embryo and appears as in Fig. 8. It is a more rounded structure than is the notochord of the toad embryo at a corresponding stage of development (Fig. 3), yet it, too, is closely connected with the endodermal layer of cells forming the mid-dorsal wall of the archenteron. As shown in Fig. 8, the lateral mesoderm and the endoderm of the archenteric wall are connected for some distance on either side of the notochord. The cells of both of these tissues have the same general characteristics, and there is no sharp distinction between them as in the embryo of *Bufo*. As all of the cells in the dorsal part of the embryo have the same power of staining, it is not easy to follow the changes that take place, even with an abundance of material for study. Not until I had made camera drawings of a complete series of sections through the posterior region of an unusually favorable embryo was I able to tell with certainty how the notochord is formed. Four of these drawings (from the same embryo as Fig. 8) are reproduced in Figs. 9-12. For the sake of clearness only the dorsal wall of the archenteron and the notochord are shown. In all of the sections the mesoderm is entirely cut off from the notochord, and also from the endoderm beneath it.

A short distance in front of the region shown in Fig. 8, almost

all of the pigment in the mid-dorsal wall of the archenteron is found to be massed in the outer edges of a very few cells which are entirely cut off from the other cells of the archenteric wall and are attached to the lower surface of the notochord (Fig. 9). These few cells are undoubtedly comparable to the layer of chorda-endoderm found in the mid-dorsal wall of the archenteron



FIGS. 8-12. Serial sections from the posterior to the middle region of an embryo of *Rana palustris* in which the medullary folds are closing.

in the toad embryo, and, therefore, the same term may fitly be applied to them. More anteriorly (Fig. 10) there is a noticeable upward bend in the mid-dorsal wall of the archenteron, and it appears as if the notochord with the chorda-endoderm cells is either pulling in or being pushed in from bordering the archenteric cavity, while the cells of the dorsal wall of the archenteron

on either side of the notochord are coming together under the notochord. A few sections beyond (Fig. 11), the notochord is almost entirely cut off from the archenteron, as only one or two heavily pigmented cells lie between the two parts of the dorsal endoderm. In the middle region of the embryo (Fig. 12), the endodermal cells have united under the notochord and the notochord is a rounded chord of cells entirely separated from the surrounding tissues.

In *Rana palustris*, therefore, as well as in *Bufo lentiginosus*, the notochord is composed entirely of mesoderm in the anterior part of the embryo, and of mesoderm and chorda-endoderm in the posterior region. The early stages in the formation of the notochord are very similar in the two species; but in *Rana* there is no upward growth of yolk cells as in *Bufo* to form the permanent dorsal wall of the archenteron.

Most of the embryologists who have studied the early development of the Urodela agree with Jordan (10) who describes the formation of the notochord in the common newt as follows: "The cells of the median dorsal wall of the archenteron assume a somewhat columnar form and are gradually pushed up and pinched off until they are completely separated from the endoderm and come to lie above it in the mid-line." This view is held by Hertwig (7), Scott and Osborn (20), Field (5), Eycleshymer (4), Brachet (2), and Schwink (19).

Lwoff (13) is, perhaps, the most prominent of those who oppose this view. In his study of *Axolotl*, Lwoff finds that the mesoderm and the notochord are derived from cells invaginated from the surface of the egg at the blastopore rim, and he states: "Bei den Urodeelen bildet sich die dorsale Wand des Darmes, ebenso wie bei *Petromyzon*, verhältnissmäßig spät, nämlich nachdem die Chorda sich von den seitlichen Mesodermplatten gesondert hat. Die Entodermzellen wachsen von rechts und links einander entgegen, vereinigen sich unter der Chorda und bilden aufsolche Weise die dorsale Wand des Darmes." This description of the manner in which the permanent dorsal wall of the archenteron is formed in the *Axolotl* agrees remarkably well with the results of my investigations on *Bufo*. Lwoff's summary of the results of his study of the Anura based on an

investigation of the early development of *Rana*, is in part as follows : " Bei den Anuren liegen insofern anderen Verhältnisse vor, also hier die Zellen, welche die dorsale Wand des Darmes bilden, von Anfang an vorhanden sind ale eine Zellenreihe und zwar als eine untere Zellenreihe jener Anlage, aus welcher die Chorda entsteht." Lwoff and I are therefore in accord in believing that in *Rana* there is no upward growth of the yolk cells from the lateral walls of the archenteron to form the mid-dorsal wall.

There is great diversity of opinion concerning the manner of the formation of the notochord in the Anura ; and, considering the careful work that has been done in this line, it seems highly probable that the process is not as uniform in this group as it is in the Urodela.

Goette (6), from his study of the development of *Bombinator igneus* concludes that in this species a central chord of mesoblast in the mid-dorsal region of the embryo separates from the two lateral sheets to form the notochord. This view is supported by the later investigations of Schultze (18), and Morgan (15) who worked on different species of *Rana*.

In a paper on the development of the middle germ layer in *Rana temporaria*, Hertwig (8) gives a number of figures of the posterior part of the embryo that bear a striking resemblance to those I have drawn of a similar region in the embryo of *Bufo lentiginosus*. Hertwig believes, however, that the entire notochord in the Anura as well as in the Urodela, is derived from a chorda-entoblast which at the sides of the archenteron pass into the endoderm cells forming the lateral walls. Field (5), from his investigations on *Rana temporaria* and on *Bufo vulgaris*, agrees with Hertwig regarding the manner of formation of the notochord, as do Robinson and Assheton (17) who worked on *Rana temporaria*. Balfour (1) also inclines to the same opinion, although he states that his evidence for so doing is not entirely conclusive.

As a result of his study of the early development of *Bombinator igneus*, Perenyi (16) advances still another theory regarding the formation of the notochord. He states that, when the blas-topore closes in, " die vertikal nach innen vordringenden Zellen

der Deckzellen, welche zwischen beiden Teilen des Mesoderms liegen einander berühren und sich auf der dorsalen Seite von den äussersten Zellen abzuschnüren beginnen." In this way a rod of cells is cut off from the inner layer of ectoderm to become the notochord. I know of no other investigator whose results agree with those of Perenyi.

The results which Schwink (19) has obtained from his investigations on *Rana temporaria* and *Bufo vulgaris* are very similar indeed to those which I have recorded in the present paper for *Rana palustris* and *Bufo lentiginosus*. According to Schwink, the anterior portion of the notochord in *Rana temporaria* is entirely mesodermal in origin, while the posterior part has added to it a single layer of chorda-endoderm from the dorsal wall of the archenteron, the endoderm cells at the side of the notochord growing under and uniting in the mid-dorsal line. In *Bufo vulgaris* Schwink finds that the dorsal wall of the archenteron is composed of deeply pigmented cells which, at the sides of the archenteron, pass into the larger yolk cells, although he states that in some cases it appears "dass die hier liegenden Entoblastzellen aus dem bisherigen Verband scheiden um in den Mesoblast aufgenommen zu werden." Concerning the formation of the dorsal wall of the archenteron in the posterior part of the embryo Schwink states that, "hier von beiden Seiten Darmentoblastzellen gegen die Mittellinie streben und dass dadurch Zellen, die vorher den Darm dorsal auskleideten, mit zur Bildung der Chorda verbraucht werden." This agrees exactly with what I have found to occur in the posterior region of the embryo of *Bufo lentiginosus*.

Brauer's (3) studies on the development of the Gymnophiona show that, in the posterior region of the embryo, the upper wall of the archenteron is at first formed of cells which have been invaginated from the surface. These "animal cells" are sharply marked off from the yolk or "vegetative cells" which form the side walls of the archenteron. In the anterior part of the embryo, the archenteron is extended by its connection with the segmentation cavity which is bounded entirely by yolk cells. At an early stage of development, therefore, the dorsal wall of the archenteron in the anterior region of the embryo is composed of vegetative cells, while in the posterior region it is formed of cells invaginated

from the surface as I have found to be the case in the embryo of *Bufo lentiginosus*. At a later stage of development, vegetative cells grow up from the sides of the archenteron, and gradually cover up the invaginated animal cells which now form an unbroken sheet of mesoderm across the dorsal wall of the archenteron. A portion of this mesoderm in the mid-dorsal line is subsequently cut off from the lateral mesoderm to form the notochord.

In the posterior region of the embryo of *Bufo lentiginosus* a portion of the dorsal plate of cells which forms the mid-dorsal wall of the archenteron becomes cut off from the rest of the layer to be added to the notochord. If we attempt to trace the origin of this dorsal plate, we find that it is composed of cells invaginated from the surface of the egg before there was any division of the cells into ectoderm, mesoderm and endoderm. These invaginated cells form a part of the upper wall of the archenteron for a comparatively short period of development only, and those of the cells that are subsequently added to the splanchnic mesoderm soon lose their identity entirely, and cannot be distinguished in any way from the other cells of the mesoderm. The later history of the chorda-endoderm cells I have not followed.

As the endoderm cells that grow up from the sides of the archenteron and meet under the notochord are unquestionably derived from the yolk portion of the egg, the archenteron eventually becomes lined throughout its whole extent with yolk cells, and, therefore, the result is the same as if the archenteron was originally formed by a splitting between yolk cells as is believed to be the case by Robinson and Assheton (17), Houssay (9) and Moquin-Tandon (14).

According to Morgan, Wilson (21), Eycleshymer and others, there is an invagination of surface cells at the dorsal lip of the blastopore during the gastrulation of the frog's egg, and these invaginated cells come to form a part, if not all, of the dorsal wall of the archenteron in the posterior region of the embryo. In subsequent development, as the studies of Schwink and of myself show, these invaginated cells are not covered over by an upward growth of yolk cells from the lateral walls of the archenteron as is the case in the toad embryo. A few of these cells

are added to the notochord, the rest, as far as I have been able to determine, remain as part of the permanent dorsal wall of the archenteron. I have never seen a section of an embryo that would warrant my stating that some of these cells become added to the mesoderm, although in the posterior region of the embryo endoderm and mesoderm are connected for a much longer time than they are in the embryo of *Bufo*.

LITERATURE.

1. **Balfour, F. M.**
'80 Comparative Embryology. Vol. II. The Macmillan Company, New York, 1880.
2. **Brachet, A.**
'02 Recherches sur l'ontogénèse des Amphibiens, Urodèles et Anoures. Archiv. de Biologie. T. XIX., 1902.
3. **Brauer, A.**
'97 Beiträge zur Kenntniss der Entwicklungsgeschichte und der Anatomie der Gymnophionen. Zool. Jahrbüch, Bd. X., 1897.
4. **Eycleshymer, A. C.**
'95 The Early Development of *Amblystoma*, with Observations on Some Other Vertebrates. Journ. Morph., Vol. X., 1895.
5. **Field, H. H.**
'95 Bemerkungen über die Entwicklung der Wirbelsäule bei den Amphibien; nebst Schilderung eines abnormalen Wirbelsegmentes. Morph. Jahrbüch, Bd. XXII., 1895.
6. **Goette, A.**
'75 Die Entwicklungsgeschichte der Unke. Leipzig, 1875.
7. **Hertwig, O.**
'81 Die Entwicklung des mittleren Keimblattes der Wirbelthiere, I. Jena, 1881.
8. **Hertwig, O.**
'83 Die Entwicklung des mittleren Keimblattes der Wirbelthiere, II. Jena, 1883.
9. **Houssay, F.**
'90 Études d'embryologie sur les vertébrés. Archiv. de Zool. Exper. et Génér., T. VIII., 1890.
10. **Jordan, E. O.**
'93 The Habits and Development of the Newt. Journ. Morph., Vol. VIII., 1893.
11. **King, H. D.**
'01 The Maturation and Fertilization of the Egg of *Bufo lentiginosus*. Journ. Morph., Vol. XVII., 1901.
12. **King, H. D.**
'02 The Gastrulation of the Egg of *Bufo lentiginosus*. American Naturalist, Vol. XXXVI., 1902.
13. **Lwoff, B.**
'94 Die Bildung der primären Keimblätter und die Entstehung der Chorda und des Mesoderms bei den Wirbelthieren. Bull. de la Soc. Imp. des Nat. de Moscou, Bd. VIII., 1894.

14. Moquin-Tandon, G.

'76 Recherches sur les premières phases du développement des batraciens anoures.
Ann. des Soc. Nat., T. III., 1876.

15. Morgan, T. H.

'97 The Development of the Frog's Egg. The Macmillan Company, New York,
1897.

16. Perenyi, J.

'89 Die Entwicklung der Keimblätter und der Chorda in neuer Beleuchtung.
Anat. Anz., Bd. IV., 1889.

17. Robinson, A., and Assheton, R.

'91 The Formation and Fate of the Primitive Streak with Observations on the
Archenteron and Germinal Layers of *Rana temporaria*. Quart. Journ. micr.
Sci., Vol. XXXII., 1891.

18. Schultze, O.

'88 Die Entwicklung der Keimblätter und der Chorda dorsalis von *Rana fusca*.
Zeitschr. f. wiss. Zoöl., Bd. XXVII., 1888.

19. Schwind, F.

'89 Ueber die Entwicklung des mittleren Keimblattes und der Chorda dorsalis
der Amphibien. Munchen, 1889.

20. Scott, W. B., and Osborn, H. F.

'79 On some Points in the Early Development of the Common Newt. Quart.
Journ. micr. Sci., Vol. XIX., 1879.

21. Wilson, H. V.

'00 Formation of the Blastopore in the Frog's Egg. Anat. Anz., Bd. XVIII.,
1900.